

CONTROLLED POLLINATION OF *EUCALYPTUS*.

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(Plate vi.)

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Synopsis.

Successful cross-pollination in several pairs of *Eucalyptus* species and the methods employed, are recorded.

Self-fertility has been found in all species examined, but possibly some self-sterile individuals exist.

Attempts at wide crossing were unsuccessful.

INTRODUCTION.

At various times the possibility of hybridization between species in the genus *Eucalyptus* has been discussed and in the taxonomic treatment of the genus some described species have been considered as possible hybrids by both Maiden and Blakely. There has been little if any record, however, of controlled hand-pollination within the genus apart from some successful attempts which have been made by Brett (1949) and also Gilbert and Martin (1949) in Tasmania.

Recently abundant evidence has been produced by Brett (1949) and Pryor (1950) to show that under field conditions hybrids between several well-established species can be found quite easily. The evidence for this has been principally morphological and that secured from progeny tests. A rigorous demonstration of the contentions requires that, in addition, the supposed hybrids shall be synthesized by controlled pollination. A critical examination of the methods of pollination, both artificial and natural, is also necessary in many other ways. It is important to know the extent of self-sterility in the genus, the amount of out-crossing in self-fertile species, and similar things before we can hope to understand the complex genetic structure within the genus. This genetic knowledge seems essential before *Eucalyptus* taxonomy can be placed on a scientific basis. Barber (1950) has strongly emphasized this need for genetic knowledge and the experimental method in taxonomy in general in Australia, while some knowledge of the mechanism of reproduction is a prerequisite to breeding *Eucalyptus* for economic or aesthetic needs.

POLLINATION METHODS.

The method of reproduction in the species examined is one which aids cross-fertilization of each individual flower. Usually the pollen is shed soon after the operculum falls, and from most anthers often within the first twenty-four hours. However, to a varying degree some inner anthers retain pollen, which is not shed until later, and then as they unfold more or less brush the stigma and no doubt encourage self-pollination if there has not already been successful pollination. The stigma in most cases is apparently not receptive until several days after the operculum falls, and then it seems to remain receptive, often for four or five days, and sometimes up to ten days. This means that the flowers are protandrous, which is common in many genera. In a few flowers examined a little pollen has been shed before the operculum falls, but the extent to which this occurs cannot be determined without more examination. Apparently Mueller had this in mind when he expressed the opinion that cross-pollination is unlikely in a genus where a calycine lid guards the stigma.

There seems to be little wind-pollination and undoubtedly the genus is principally insect-pollinated. The arrangement of the stamens, stigma and nectaries, as well as the time of maturation of pollen and stigma, favours cross-pollination in any one flower

with the help of insects. The flowers when open attract many kinds of insects, and in some localities birds may be important.

Emasculation is a simple matter and may be effectively carried out, just before the operculum falls, by cutting through the tissue just below the staminal ring but above the top of the ovary. With most species the stamens and operculum come away freely leaving the stigma uninjured, but in a few species examined the stigma is tightly enclosed by the filaments and the stamens and operculum cannot be removed, if the stigma is to remain undamaged, without a second cut longitudinally to free them. If this has to be done the speed of emasculation is greatly reduced.

The method of preparing flowers for controlled pollination is quite easy; the principal experimental difficulty is to prevent burning of the prepared flowers after bagging. Where there is no free air circulation, such as by the use of brown paper bags, or alternatively transparent cellulose bags, the flowers and young stems often die. This is particularly the case with those species which flower in mid-summer or autumn. Burning can generally be prevented by using muslin bags, but these possibly permit the entry occasionally of stray pollen which, while of little importance in a general breeding programme, may be troublesome if some critical examination is being made of reproductive methods. While not easy with large trees, shading in some way is necessary in these cases. Improved methods for this work should result with more investigation.

After pollination it is usually apparent in three months whether the fruit has set. Unfertilized fruit does not as a rule develop and often falls. With most species examined the fruit is ripe in ten to twelve months after pollination and may be harvested then. The seed may be developed and viable a little earlier, but the fruit is often difficult to open if not fully ripe and the seed may not be plump.

Very little fruit is set and almost no fertile seed obtained from flowers that have been emasculated and placed in muslin bags, showing that wind-pollination is practically absent and in fact the occasional pollination which occurs probably results from a very small insect, such as a thrip, a chance dropping of pollen or perhaps some pollen shed before the stamens were removed.

In most of the trials fresh pollen was used from flowers opening at the time the prepared flowers of the seed parents were receptive. Two pollinations, seven days apart, were made in most cases. There is little doubt that pollen can be stored, however, and successful germination was obtained with pollen that had been stored a month in a desiccator at 50% humidity and at about 35°F. Germination tests can be made successfully with many species, using a 10% honey solution and placing in an incubator at 70°F. The most striking feature from preliminary tests of this kind was the great variability in germination percentage between pollen from flowers on different branches of the same tree and even at times between different flowers on the same branch. Germination percentage declines after three months' storage in a refrigerator. Dry storage at room temperature results in quicker loss of power to germinate.

These preliminary tests indicate that separation of flowering times by as much as three months, and probably more, is not likely to be an obstacle to successful cross-pollination if the species are compatible and no barriers to successful fertilization exist. Much more study of the possibilities in this direction is necessary.

INTERSPECIFIC HYBRIDIZATION.

After the basic method had been successfully determined as above, a series of trials was made using principally *E. bicostata* and *E. Maidenii* as female parents. These trials aimed to assess the possibilities of interspecific hybridization and to learn where general barriers might exist within the genus. Because it was necessary, for the reasons described, to use muslin bags the chance of some occasional stray pollen fertilizing a flower could not be entirely removed. This means that some of the low numbers of fertile seed obtained must be regarded with suspicion. Where there have been substantial quantities of seed obtained—about fifty or more from ten to twenty flowers—there is no doubt that the pollination has been successful, and this is at once

obvious from the seedlings because of the marked differences obtained in the lots of seedlings from the same female parent but with different pollen parents.

Table 1 shows the results obtained with the species mentioned as female parents.

Keeping in mind the limitations of the data due to the failure to exclude all possibility of chance pollination or perhaps occasional self-pollination before the operculum falls, the trend is at once clear in compatibility in cross-pollination.

TABLE 1.

| Seed Parent. | Pollen Parent. | Number of Flowers Treated (Approx.). | Number of Fruit. | Number of Plants Raised. |
|---|--|---|---------------------|---|
| <i>E. Maidenii</i> F. Muell. | <i>E. bicostata</i> Maiden, Blakely & Simmonds. | 40 | 35 | 90 |
| | <i>E. rubida</i> Deane & Maiden .. | 12 | 10 | 35 |
| | <i>E. Blakelyi</i> Maiden | 10 | 7 | 33 |
| | <i>E. Macarthuri</i> Deane & Maiden .. | 10 | 4 | 10 |
| | <i>E. Maidenii</i> F. Muell. | 6 | 4 | 16 |
| | <i>E. cinerea</i> F. Muell... .. | 10 | 3 | 16 |
| | <i>E. ficifolia</i> F. Muell. | 10 | 2 | 1* |
| | † <i>E. Bridgesiana</i> R. T. Bak. .. | 10 | 1 | — |
| <i>E. bicostata</i> Maiden, Blakely & Simmonds (Horse yards). | <i>E. bicostata</i> Maiden, Blakely & Simmonds (another tree). | 15 | 12 | Harvested too early. Germination except for a few plants a failure. |
| | <i>E. Maidenii</i> F. Muell. | 15 | 12 | |
| | <i>E. Maidenii</i> F. Muell. | 15 | 10 | |
| | <i>E. Maidenii</i> F. Muell. | 10 | 7 | |
| | <i>E. Blakelyi</i> Maiden | 10 | 2 | |
| | <i>E. ficifolia</i> F. Muell. | 10 | 1 | |
| | <i>E. cinerea</i> F. Muell... .. | 6 | — | |
| | Self | 8 | — | |
| <i>E. bicostata</i> Maiden, Blakely & Simmonds (Acton Park). | Self | 15 | 15 | Not yet raised. |
| | <i>E. Maidenii</i> F. Muell. | 10 | 7 | |
| | † <i>E. Bridgesiana</i> R. T. Bak. .. | 10 | 1 | |
| | ‡ <i>E. delegatensis</i> R. T. Bak. .. | 6 | — | |
| | <i>E. Macarthuri</i> Deane & Maiden .. | 8 | — | |
| <i>E. Blakelyi</i> Maiden (Capital Hill). | <i>E. Blakelyi</i> Maiden | 6 | 4 | 12 |
| | <i>E. rubida</i> Deane & Maiden .. | 5 | 2 | 3 |
| | <i>E. cinerea</i> F. Muell... .. | 5 | 1 | — |
| | <i>E. melliodora</i> A. Cunn. | 10 | — | — |
| | Control (not poll.) | 7 | — | — |
| <i>E. Blakelyi</i> F. Muell. (Acton). | <i>E. cinerea</i> F. Muell... .. | 12 | 8 | 9 |
| <i>E. cinerea</i> F. Muell. (Arboretum). | <i>E. Blakelyi</i> F. Muell. | 6 | 5 | 21 |

* Appears to be pure *E. Maidenii*.

† = *E. Stuartiana* F. Muell.

‡ = *E. gigantea* Hook. f., non Dehnh.

Highly successful cross-pollination is achieved by using pollen of the same species and of more or less closely related species, for example, *E. Maidenii* with *E. bicostata*, *E. Blakelyi*, *E. Macarthuri* and *E. cinerea*, which are all in the anther group of Blakely, Macrantherae; whereas *E. ficifolia* and *E. Maidenii*, *E. bicostata* and *E. delegatensis*, *E. Blakelyi* and *E. melliodora* are pairs in widely separated groups, and practically no setting of fruit has been obtained.

There is no doubt that successful crosses between *E. Maidenii* and *E. Blakelyi*; *E. Maidenii* and *E. cinerea*; *E. Maidenii* and *E. rubida*; *E. Maidenii* and *E. Macarthuri*; and *E. Blakelyi* and *E. cinerea* have been achieved, because of the distinct characters already developed in these progenies.

In the main, the F1 hybrids show juvenile characters intermediate between the parents.

Striking evidence of successful cross-pollination is furnished by the *E. Maidenii* × *E. Macarthuri* hybrids due to the incorporation of the highly distinctive geraniol oil of *E. Macarthuri* in the F1 hybrid after having been transmitted by the pollen of *E. Macarthuri*.

The trend is therefore clear that the more widely separated the species within the genus, the less likely that cross-fertilization can be achieved. To determine the absolute limits and whether any progeny obtained is fertile will require more experiment.

This supports the field evidence that hybrids between species within the different anther groups of Macrantherae, Renantherae and Terminales are rare or absent.

In one case, in addition to *E. Maidenii* × *E. bicostata* the reciprocal cross has also been made between *E. cinerea* and *E. Blakelyi*. The hybrid progeny with *E. cinerea* the seed parent, and the other with *E. Blakelyi* the seed parent, is intermediate, at the ten pairs of leaves stages, in morphology between the parents. Both progenies are closely similar to each other but at the same time quite distinct from open-pollinated seedlings of each parent.

SELF STERILITY.

Krug and Alves (1949) have stated that with the species of *Eucalyptus* examined in Brazil they believe them self-sterile. This is contrary to the condition revealed in the species examined here. The species critically examined are substantially different from those used by Krug and Alves, the latter dealing with species common on the north coast of New South Wales, in the coastal climate, while the species which have been subject to experiment here grow naturally on the cold Southern Tablelands. It is clear that those on which the experiments have been made can successfully self-pollinate because, if flowers are bagged before the operculum falls (and in one experiment with *E. Maidenii* double bags were used consisting of an outer one of muslin and an inner one of brown paper), good fruit is set, giving fertile seed. On the other hand, if the flowers were emasculated and then bagged, fruit did not set and no fertile seed was obtained. In a further experiment with the three species, *E. Maidenii*, *E. Blakelyi* and *E. bicostata*, bagging, emasculation and hand-pollination with pollen of the same plant were followed by good setting of fruit and production of ample fertile seed. This particular experiment on *E. bicostata* was duplicated on separate trees. It led to an interesting case of individual variation which must be examined in more detail. In the second tree, unlike the first, flowers bagged before opening did not set seed, therefore apparently not selfing. In addition, flowers emasculated and bagged did not set when self-pollinated with other flowers of the same tree, but similarly emasculated and bagged flowers pollinated with pollen from another tree of *E. bicostata* set ample fruit.

This suggests that either the particular tree is self-sterile or produced defective pollen in 1949, or perhaps does so always. Further examination is necessary to find the complete explanation, but in any one of the three cases there could be a factor of considerable importance in the production of hybrid seed in quantity.

SUMMARY.

Simple trials show that many species of *Eucalyptus* are protandrous, entomophilous, and with a mechanism to aid cross-fertilization of each flower but some capacity for self-pollination if crossing fails.

Emasculation is easy and controlled pollination simple, provided the parents are fairly closely related within the genus. Successful controlled pollinations have been achieved with several pairs of species, and in two cases reciprocal crosses have been made.

Absolute barriers and the limit of crossing, although indicated, have yet to be accurately determined. Self-fertility exists in a number of species.

Burning of flowers and stems following bagging must be carefully guarded against.

References.

- BARBER, 1950.—Taxonomy. *Aust. Journ. Sci.*, 12: 184.
 BRETT, 1949.—*A.N.Z.A.A.S.*, Hobart, xxviii: 149.
 GILBERT and MARTIN, 1949.—Unpublished communication.
 KRUG and ALVES, 1949.—Eucalyptus Improvement. *Journ. Hered.*, 40: 133-139, 143-150.
 PRYOR, 1950.—A Hybrid Eucalyptus. *PROC. LINN. SOC. N.S.W.*, 75: 96-98.

EXPLANATION OF PLATE VI.

Fig. 1.—Progeny obtained from hand-pollinated capsules from the one tree of *E. Maidenii*. The four plants at the left are the result of cross-pollination with *E. rubida*. Central four plants, cross-pollination with *E. cinerea*. The four plants on the right, pollination with *E. Maidenii* from another tree of the same species. The variation in population in form clearly indicates the success of the controlled pollination.

Fig. 2.—Progeny obtained from controlled pollinations on a single seed parent individual of *E. Maidenii*. The four plants on the left are the result of pollination with *E. Blakelyi* pollen; the central four, *E. bicostata*, and the four plants on the right are from open-pollinated capsules on the same tree. The marked variation in the form of the progeny as well as other characteristics, such as colour, not apparent in the photograph, indicates the success of the pollination.

Fig. 3.—Progeny from a seed parent of *E. Maidenii*. The four plants on the left are from open-pollinated capsules on the parent tree. The four plants on the right are as the result of hand-pollination with *E. Macarthuri*. Characters intermediate with *E. Macarthuri* are apparent in the more tapering leaves, particularly noticeable in the first and third individuals of the group. The unmistakable presence of geraniol in these individuals is striking confirmation of the hybridization.

Fig. 4.—The four plants on the left were obtained from a *E. cinerea* seed parent after crossing with *E. Blakelyi* by hand. The four on the right were raised from seed from open-pollinated flowers. The intermediate characters of the hybrid population are at once apparent.

Fig. 5.—The four plants on the left were obtained as the result of hand-pollination of a tree of *E. Blakelyi* with *E. cinerea*. The progeny is intermediate. The four plants at the right were the result of open-pollination on the same tree. This is a reciprocal cross of species in Fig. 4, but not with the same individuals.